

additional studies will be required to elucidate whether the spectrum of potential hosts might include other farm or companion animals, and whether the virus might be able to infect humans.

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## New Chimeric Porcine Coronavirus in Swine Feces, Germany, 2012

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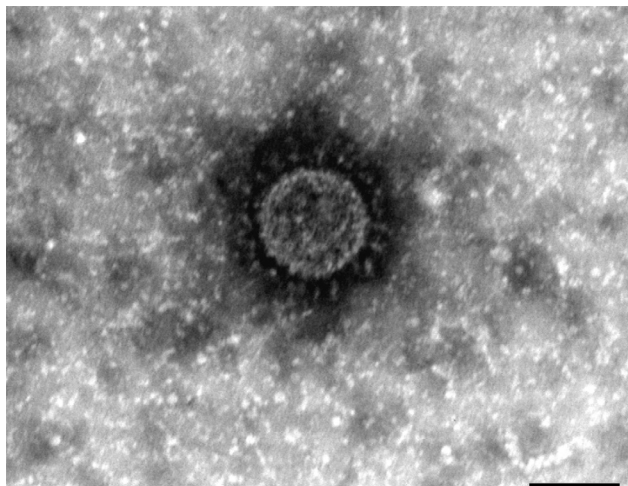
**To the Editor:** Porcine epidemic diarrhea virus (PEDV) and transmissible gastroenteritis virus (TGEV) can cause severe enteritis in pigs accompanied by diarrhea, vomiting, and dehydration. Clinical signs are most prominent in young suckling pigs, in which high mortality rates are common. As seen in recent porcine epidemic diarrhea outbreaks in the United States and Asia, the effect on the pig industry can be tremendous.

Recently, Boniotti et al. (1) reported detection and genetic characterization of swine enteric coronaviruses (CoVs) circulating in Italy during 2007–2014. Characterization was based on sequencing and phylogenetic analyses of spike genes of TGEV and PEDV isolates. This study also reported a new recombinant CoV strain with a TGEV backbone and a PEDV spike gene (SeCoV/Italy/213306/2009; KR061459), which was identified as a swine enteric CoV (SeCoV). This chimeric virus presumably resulted from a recombination event.

Accompanying a study of recent porcine epidemic diarrhea cases in Germany caused by a new PEDV Indel strain (2), we retrospectively analyzed fecal samples from pigs that showed typical clinical symptoms of a PEDV infection. The sample set included fecal material collected from a farm in southern Germany on which an episode of diarrhea among pigs occurred in 2012. This material was shown by electron microscopy to contain CoV-like particles (Figure), but showed negative results by reverse transcription PCRs specific for the PEDV nucleocapsid gene.

Subsequent metagenomic analyses resulted in the full-genome sequence of a swine enteric CoV (SeCoV/GER/L00930/2012). We found a sequence showing high similarity (99.5% identity) with the TGEV/PEDV recombinant reported by Boniotti et al. (1). Network analysis of complete genome sequences of similar CoVs underline the chimeric nature of the genome between TGEV and PEDV genome sequences (online Technical Appendix Figure,

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**Figure.** Electron micrograph of a new chimeric swine enteric coronavirus (SeCoV/GER/L00930/2012), Germany, 2012. Scale bar indicates 100 nm.

panel A, <http://wwwnc.cdc.gov/EID/article/22/7/16-0179-Techapp1.pdf>). The chimeric nature of the virus strain was confirmed by RT-PCR with primers spanning possible recombination sites and analysis of overlapping reads from next-generation sequencing.

Annotation of the sequence of SeCoV/GER/L00930/2012 performed on the basis of SeCoV/Italy/213306/2009 identified a similar putative coding sequence with a TGEV backbone and a spike coding sequence similar to that for PEDV (online Technical Appendix panel B). Downstream of the spike protein-coding open reading frame (ORF), an additional hypothetical ORF was identified in both SeCoV sequences. The coded amino acid sequences (27 aa in the virus from Germany and 30 aa in the virus from Italy) resembled an N- and C-terminally truncated TGEV nonstructural protein 3a. The difference of 3 aa between the 2 strains is the result of a 10-bp deletion at the 3'-end of the hypothetical ORF, which shifted the stop 3 codons to the 5'-end (online Technical Appendix Figure, panel B) in SeCoV/GER/L00930/2012. This deletion is apparently located within the potential 3' recombination site (online Technical Appendix Figure, panel B).

It is tempting to speculate that SeCoV/Italy/213306/2009 is a precursor of SeCoV/GER/L00930/2012, and that other members of this novel genotype are still undetected. These viruses might be targets of secondary mutation and recombination events. Therefore, more chimeric CoVs should be identified to determine the potential origin of the recombination event.

In conclusion, we detected an enteric CoV that resembled the TGEV/PEDV chimeric virus reported by Boniotti et al. (1). Although these findings support the notion that CoV genomes are subject to mutations and recombination

events, problems in disease diagnosis can be foreseen. In countries where porcine epidemic diarrhea, transmissible gastroenteritis, or both of these diseases are reportable, correct diagnosis and reporting might be difficult. Thus, diagnosticians should be aware of possible recombinants of swine CoVs. Diagnostic problems can be prevented by use of a double-check strategy with techniques specific for different genome regions. Apart from diagnostic obstacles, the effect of virus recombinations in terms of virulence and organ tropism is unknown and needs further investigations.

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## Colistin-Resistant *mcr-1*-Positive Pathogenic *Escherichia coli* in Swine, Japan, 2007–2014

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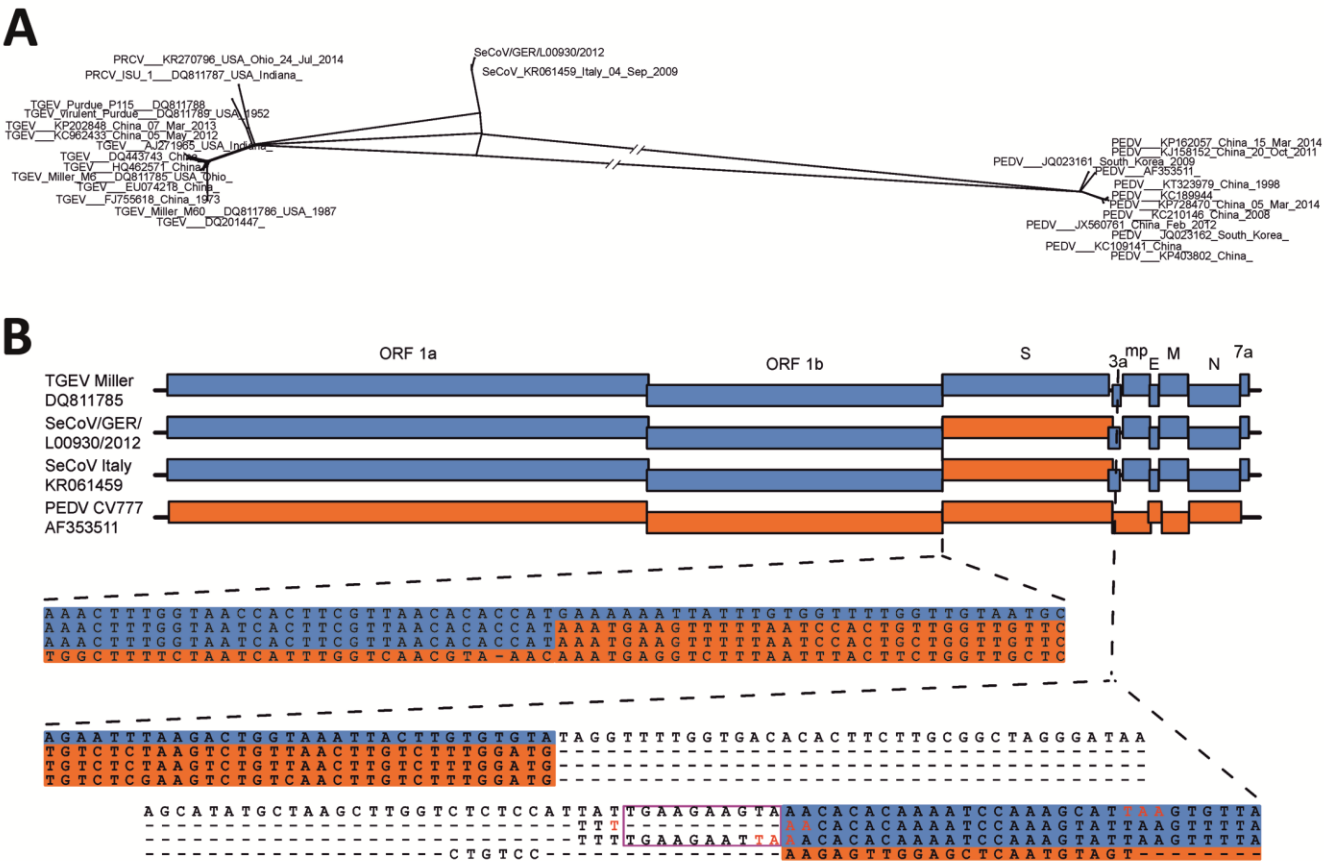
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**To the Editor:** Colistin is an old-generation antimicrobial agent; however, because it is one of the few agents that remain effective against multidrug-resistant gram-negative bacteria (e.g., carbapenem-resistant *Pseudomonas aeruginosa* and *Enterobacteriaceae*), its clinical usefulness is being increasingly recognized (1). Previous reports have described the mechanisms of colistin resistance (2) as being chromosomally mediated and not associated with horizontal gene transfer. However, from 2011 through 2014, a plasmid-encoded colistin-resistance gene, *mcr-1*, was identified in colistin-resistant *Escherichia coli* isolated in

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## Technical Appendix



**Technical Appendix Figure.** Analysis of a new chimeric swine enteric coronavirus (SeCoV/GER/L00930/2012), Germany, 2012. A) Network analysis of complete genomes of SeCoVs and related coronaviruses. PRCV, porcine respiratory coronavirus; PEDV, porcine epidemic diarrhea virus; TGEV, transmissible gastroenteritis virus. B) Top: schematic representation (not to scale) of 2 chimeric viruses and their putative progenitors. Sequences most related to progenitor strain TGEV Miller are indicated in blue, and sequences most related to PEDV CV777 are indicated in red. Protein designations are according to the International Committee on Taxonomy of Viruses. ORF, open reading frame; S, spike; 3a, accessory proteins encoded by ORF3; mp, alphacoronavirus-specific accessory membrane protein amp; E, envelope; M, membrane; N, nucleocapsid; 7a, accessory proteins encoded by ORF7. Bottom: alignments of the putative recombination sites. A deletion in SeCoV from Germany is indicated by the purple box, and stop codons of the hypothetical nonstructural protein 3a coding ORFs are indicated in red.